

# Effects of Low Concentrations of O<sub>3</sub> on Net Photosynthesis, Dark Respiration, and Chlorophyll Contents in Aging Hybrid Poplar Leaves<sup>1</sup>

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## ABSTRACT

Chronic exposure of hybrid poplar (*Populus deltoides* × *trichocarpa*) plants to low concentrations of ozone had negative impact upon net photosynthetic capacity, dark respiration, and leaf chlorophyll contents. Exposure to as much as 0.20 microliters per liter O<sub>3</sub> had no immediate effect on net photosynthesis ( $P_n$ ), but chronic exposure to 0.125 or 0.085 microliters per liter had a number of gradual effects on CO<sub>2</sub> exchange. These included increased dark respiration and consequently increased light compensation points in very young leaves (4–6 days old); and decreased  $P_n$ , leaf chlorophyll *a* and *b* contents, light saturation points, and apparent quantum yields in leaves 10 to 70 days old. Decreased  $P_n$  was partially due to accelerated aging in leaves exposed to O<sub>3</sub>, and light-saturated  $P_n$  was linearly related to chlorophyll *a* + *b* contents. Differences in light-saturated  $P_n$  between control and O<sub>3</sub>-treated leaves of the same age were mostly due to photosaturation in O<sub>3</sub>-treated leaves and to a much lesser extent to lowered apparent quantum yields. Also, since  $P_n$  and dark respiration were most affected by O<sub>3</sub> at different leaf ages, distinct modes of action are suggested. The effects of leaf aging on CO<sub>2</sub> exchange were considerable, but typical of other species. However, careful monitoring of the interacting effects of leaf age and pollutant exposure was needed in order to characterize the impact of chronic O<sub>3</sub> exposure upon CO<sub>2</sub> exchange.

Ozone is currently the most ubiquitous and damaging gaseous air pollutant in industrialized regions of the world. Ozone is phytotoxic at high concentrations (>0.20  $\mu\text{l l}^{-1}$ ), but until recently little was known about the effects of the lower concentrations (<0.15  $\mu\text{l l}^{-1}$ ) which are much more typical of concentrations in ambient air, even in most urban locales. Recent studies have found significant negative impact upon growth and yield of a number of species following chronic, low level O<sub>3</sub> exposure (3, 4, 7, 16, 17). Unfortunately, the mechanisms underlying such effects are not well understood. Although exposure to high concentrations of O<sub>3</sub> can have dramatic and rapid effects, such as visible leaf injury, reduced photosynthetic rates, and cell death, extrapolation from such results to the situation at lower concentrations is unrealistic, inasmuch as the sequence of events which

ensues in the latter case is probably different than in the former. Exposure to low concentrations of O<sub>3</sub> will usually not mark leaves or cause cell death, and very little is known about processes such as photosynthesis, respiration, or stomatal function under such circumstances. For these reasons, I examined certain aspects of the net CO<sub>2</sub> exchange of a hybrid poplar (*Populus deltoides* × *trichocarpa*) chronically exposed to approximately 0.08 or 0.12  $\mu\text{l l}^{-1}$  ozone. This paper presents data showing the effects of such exposures upon photosynthetic capacity, dark respiration, and Chl contents in hybrid poplar.

## MATERIALS AND METHODS

One hundred and ten cuttings (25 cm in length) of hybrid poplar (Northeastern Forest Experiment Station Clone 207; *Populus deltoides* × *trichocarpa*) were planted in a greenhouse in Ithaca, NY, on April 17, 1981. These cuttings were taken from young trees grown by the author in the field in Ithaca. Cuttings were grown in 19-L pots, in a 1:1:1 mixture (v/v) of peat, vermiculite, and soil, and were fertilized weekly with a 20:20:20 NPK mixture (Peters General Purpose 20-20-20; Peters Fertilizer Products, Fogelsville, PA). On May 5, 10 plants to be used for CO<sub>2</sub> exchange measurements were placed in each of four controlled environment growth chambers (Western Environmental, Napa, CA). Air in the growth chambers was drawn from outdoors through Purofil filters (Purofil Corporation, Chamblee, GA) which removed most of the ambient O<sub>3</sub>. Chamber conditions included 16-h photoperiods with half light intensity for the first and last half hours of each photoperiod. Day/night temperatures and relative humidities were 25/20 ± 1°C and 60/70 ± 5%, respectively. PAR at the top of the plant canopy was about 900  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and was measured with a quantum sensor (LI-COR LI-190s).

Four approximate pollutant treatments were chosen (filtered air/control, 0.04, 0.08, and 0.12  $\mu\text{l l}^{-1}$  ozone) and were assigned randomly to the four growth chambers. Ozone was generated by passing O<sub>2</sub> by a UV light source and bled into the chambers. Air in the chambers was sampled using Teflon lines, and concentrations of O<sub>3</sub> were measured by a chemiluminescent monitor (Monitor Labs model 8410E) and continuously recorded (Leeds and Northrup Speedomax M). A switching system was used to alternately monitor air quality in each chamber, for 15 min at a time, except during intensive measurements of CO<sub>2</sub> exchange. At such times, air quality in each chamber was monitored for approximately 5 min of each 20-min period, and line switching was done manually.

Plants were exposed to O<sub>3</sub> for approximately 5.5 h d<sup>-1</sup> on 62 d during the 67-d experimental period. Total treatment mean concentrations during all exposures were 0.025 (filtered air),

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0.050, 0.085, and  $0.125 \mu\text{l l}^{-1}$  ozone, respectively. Daily mean concentrations during exposures were always within  $\pm 0.01 \mu\text{l l}^{-1}$  of the respective treatment mean concentrations.

Net photosynthetic rates ( $P_n$ ) were assessed in the growth chambers for intact, individual leaves enclosed in either of two thermoelectrically cooled and heated cuvettes (Portable Environments Cuvette and Temperature Control System, Kananaskis Centre for Environmental Research, Alberta, Canada). Growth chamber air was passed through the cuvette, the air flow rate was continuously monitored, and the difference in the  $\text{CO}_2$  concentrations between the air stream entering and leaving the cuvette was continuously measured by an IR  $\text{CO}_2$  analyzer (ANARAD model AR-600), and recorded. The cuvette atmosphere was mixed constantly by a fan and tests of either  $\text{CO}_2$  or  $\text{O}_3$  concentrations found no gradients within the cuvette. The  $\text{CO}_2$  analyzer was calibrated daily in the differential mode using certified  $\text{CO}_2$  standard gases (Scott Specialty Gases, Plumsteadville, PA). During measurements of  $\text{CO}_2$  exchange, air temperature in the cuvette was  $25^\circ\text{C}$  and  $\text{CO}_2$  concentration ranged between 330 and  $340 \mu\text{l l}^{-1}$ . The inner surfaces of the cuvettes were lined with Teflon, but significant absorption of  $\text{O}_3$  still occurred. Thus, it was necessary to add additional  $\text{O}_3$  to the cuvette in order to match the  $\text{O}_3$  concentration in the growth chamber. This was done during all measurements of  $\text{CO}_2$  exchange, and  $\text{O}_3$  concentrations in the cuvettes were monitored as described above. Areas (one side) of paper replicas of sampled leaves were measured using a leaf area meter (LI-COR model LI-3000), and  $\text{CO}_2$  exchange rates were calculated on a one-sided leaf area basis.

Inasmuch as the objectives of this study included characterizing the interacting effects of leaf age and  $\text{O}_3$  exposure on  $\text{CO}_2$  exchange, measurements of  $P_n$  and dark respiration were made on leaves of various ages in all treatments. Leaf ages were monitored by recording the emergence date and node position of each newly emerged leaf throughout the study. Net photosynthetic rates followed a similar daily pattern regardless of age or treatment: they increased rapidly following the beginning of the photoperiod and declined very gradually from midafternoon until the end of the photoperiod.

Measurements of  $\text{CO}_2$  exchange were made and recorded at approximately midday for two leaves per day by switching from one cuvette to the other in approximately 10- to 15-min cycles. For many leaves, light response curves were generated (between 1130 and 1530 h EST) by monitoring  $\text{CO}_2$  exchange following eight or nine decreasing, and then ascending, step changes in PAR incident upon the adaxial leaf surface. Each light level was maintained until  $P_n$  had shifted and equilibrated to the new level. Apparent quantum yields were estimated from the initial slopes of the light response curves.

Because of time and equipment constraints (e.g. four treatments and two cuvettes),  $\text{CO}_2$  exchange in plants exposed to  $0.050 \mu\text{l l}^{-1}$   $\text{O}_3$  was infrequently measured. Thus, all the following results and discussion pertain only to the other three treatments.

At the conclusion of the experiment, a  $0.78 \text{ cm}^2$  leaf disc was taken from each side of the midvein in the midleaf area in six leaves (different plants) of five ages (three ages in the  $0.085 \mu\text{l l}^{-1}$  treatment) within each treatment. The two discs per leaf were combined into a composite sample and each leaf was considered a replicate sample. Leaf discs were homogenized in 80% acetone and leaf Chl *a* + *b* contents were determined for each sample according to the methods of Arnon (1).

## RESULTS

**Net Photosynthesis.** Exposure to  $\text{O}_3$  at concentrations less than  $0.20 \mu\text{l l}^{-1}$  had no immediate or short term (less than 1 d) effect on  $P_n$  (data not shown). No changes in  $P_n$  were found

following step changes in  $\text{O}_3$  concentration between 0.01 and  $0.20 \mu\text{l l}^{-1}$ , regardless of leaf age, previous treatment, direction of step change, or duration of  $\text{O}_3$  exposure (up to several hours). However, gradual changes in net photosynthetic capacity and in dark respiratory rates were observed to be influenced both by leaf age and chronic  $\text{O}_3$  exposure (Fig. 1). Changes in light-saturated  $P_n$  associated with increasing leaf age (a rapid rise followed by a gradual decline) were similar to those reported for other species (2, 8, 10). Light-saturated  $P_n$  increased rapidly until leaves were about 7 d old, reached a peak at about 11 to 16 d, and then gradually declined with increasing age (Fig. 1A). During the first 7 d,  $P_n$  of leaves chronically exposed to  $0.125 \mu\text{l l}^{-1}$   $\text{O}_3$  differed only slightly from control leaves. However, once leaves became fully expanded (after 14 d),  $P_n$  in leaves exposed to either 0.085 or  $0.125 \mu\text{l l}^{-1}$   $\text{O}_3$  was always much lower than in similarly aged control leaves. The general aging pattern was evident in all treatments, but exposure to increasing concentrations of  $\text{O}_3$  tended to accelerate the rate at which the leaf passed through its photosynthetic, and actual, life cycle ( $\text{O}_3$ -treated leaves lived approximately 10 d less than control leaves). Also,  $P_n$  increased throughout leaf expansion in control leaves and reached a maximum of  $35.7 \text{ mg dm}^{-2} \text{ h}^{-1}$  at approximately 16 d, whereas  $0.125 \mu\text{l l}^{-1}$   $\text{O}_3$ -treated leaves reach a maximum  $P_n$  of  $30.6 \text{ mg dm}^{-2} \text{ h}^{-1}$  at 11 d, prior to full expansion.

**Dark Respiration.** Age-related changes in  $R_D$  were also similar to those previously reported for other species (2). Dark respiration increased rapidly until leaves were about 6 d old (to approximately  $7 \text{ mg dm}^{-2} \text{ h}^{-1}$  in leaves exposed to  $0.125 \mu\text{l l}^{-1}$   $\text{O}_3$ ), and then rapidly declined to relatively low levels in another 10 d (Fig. 1B). For leaves 4 to 35 d old,  $R_D$  in  $0.125 \mu\text{l l}^{-1}$   $\text{O}_3$ -treated leaves was considerably higher than in leaves receiving filtered air. After 35 d,  $R_D$  in leaves in the  $0.125 \mu\text{l l}^{-1}$   $\text{O}_3$  treatment was still higher than in similarly aged leaves exposed to filtered air, but differences were small and rates in general were low.

**Light Saturation, Light Compensation, and Apparent Quantum Yield.** As leaves expanded both the light saturation point and the apparent quantum yield increased in leaves from all treatments (Fig. 2). Differences in light saturation point between control ( $0.025 \mu\text{l l}^{-1}$ ) and high  $\text{O}_3$  ( $0.125 \mu\text{l l}^{-1}$ ) treated leaves did not appear until leaves were greater than 10 d old, whereas between-treatment differences in apparent quantum yield were apparent at 5 d of age (Fig. 2, A and C). In contrast, both the light compensation points and the differences in light compensation points between treatments were at their maxima in very young leaves (4 to 5 d old), and declined with increasing age (Fig. 2B). The effect of age on light compensation can be explained by the high dark respiratory rates (Fig. 1B) and relatively low quantum yields of very young leaves. Large differences in light compensation between young control and high  $\text{O}_3$ -treated leaves were due to relatively greater dark respiration and lower apparent quantum yields of leaves in the high  $\text{O}_3$  treatment.

Once leaves were fully expanded (after 14 d), further changes in light compensation points were minimal, while apparent quantum yields and light saturation points exhibited gradual declines with increasing age (Fig. 2). Differences in apparent quantum yields between leaves exposed to filtered-air and  $\text{O}_3$  treatments were relatively stable over that period, while the difference in light saturation of leaves in the various treatments grew continuously larger.

**Light Response Curves.** The response of  $P_n$  to changes in PAR incident upon the adaxial leaf surface (Fig. 3) is determined by quantum yield at lower, nonsaturating light levels, and by gradual photosaturation at increasingly higher light levels, while the  $y$ -intercept is a function of dark respiration. The photosynthetic light response of leaves exposed to 0.025, 0.085, or  $0.125 \mu\text{l l}^{-1}$   $\text{O}_3$  for 6, 24, 38, or 58 d was due both to treatment and to leaf age, and was related to the above mentioned photobiological

<sup>3</sup> Abbreviations:  $P_n$ , net photosynthesis;  $R_D$ , dark respiration.

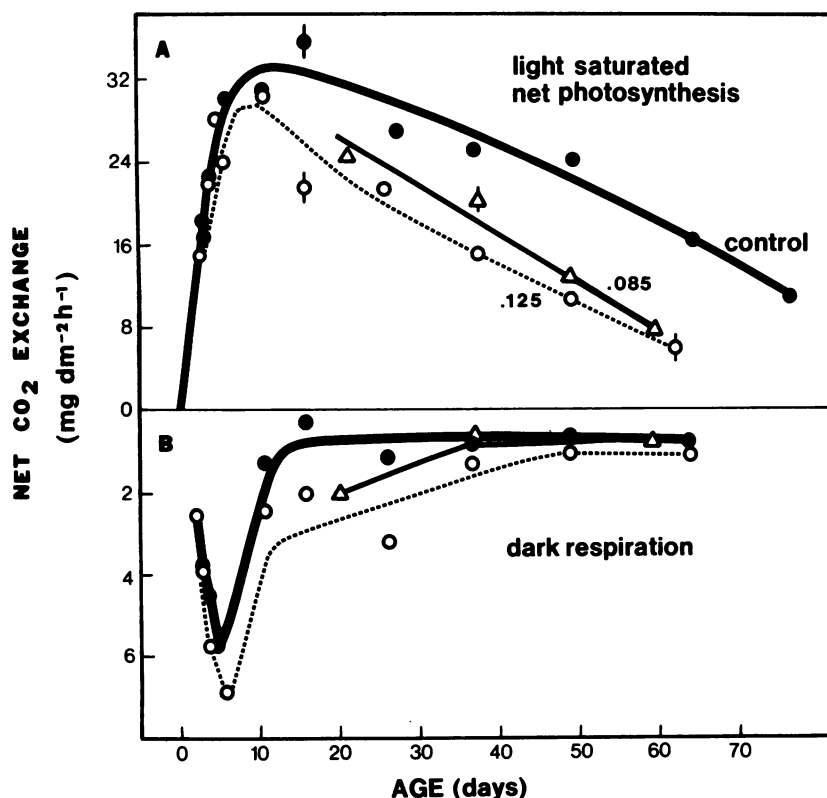


FIG. 1. Light-saturated net photosynthesis (A) and dark respiration (B) of variously aged hybrid poplar leaves chronically exposed to either 0.025 (●; control), 0.085 (Δ), or 0.125 (○)  $\mu\text{l l}^{-1}$  ozone. All points are means ( $\pm$  SE) for at least three similarly aged leaves. Where SE bars are not shown, SE fall with the symbols. This applies to all figures.

processes. In expanding leaves (6 d old), response of  $P_n$  to light was basically similar in filtered air and O<sub>3</sub>-treated leaves (Fig. 3). The only difference was a consistent (1–2  $\text{mg dm}^{-2} \text{h}^{-1}$ ) gap between the two treatments, probably due to higher dark respiration (Fig. 1) in the high O<sub>3</sub> treatment. As control leaves aged (from 24–58 d old), there was a gradual decline in the light saturation point and in  $P_n$  at that light level. In leaves of similar ages exposed to increasing concentrations of O<sub>3</sub>, those declines were more rapid. Thus, the differences in light needed for saturation and in light-saturated or maximum  $P_n$  between control and O<sub>3</sub>-treated leaves increased with increasing age. Also, differences in light-saturated  $P_n$  between control and O<sub>3</sub>-treated leaves appear to be mostly due to photosaturation of O<sub>3</sub>-treated leaves and not due to differences in the quantum yield efficiency of the photosynthetic systems.

**Leaf Chl Contents and Relationship to  $P_n$ .** Ozone treatment and leaf age had significant effects on leaf Chl contents (Fig. 4). Chl *a* contents reached a broad maxima at about 10 to 20 d in 0.125  $\mu\text{l l}^{-1}$  O<sub>3</sub>-treated leaves and at 15 to 25 d in control leaves, and then declined—gradually in control leaves and more rapidly in leaves exposed to increasingly higher concentrations of O<sub>3</sub>. Chl *b* contents in control leaves peaked at about 20 d and remained relatively stable with increasing age, while in O<sub>3</sub>-treated leaves Chl *b* peaked at 10 d and declined thereafter. At all ages greater than 10 d, Chl *a* and/or *b* contents of ozone-treated leaves were always lower than in control leaves.

Total Chl (*a* + *b*) contents of similarly aged leaves were linearly related to light-saturated  $P_n$ , regardless of treatment (Fig. 5). Under controlled conditions, a high correlation has often been shown between the Chl content of leaves and CO<sub>2</sub> uptake (5, 6). This relationship changed with increasing leaf age, as light-saturated  $P_n$  apparently became less efficient per unit leaf Chl.

## DISCUSSION

Exposure to low concentrations of O<sub>3</sub> had no immediate, direct effects on  $P_n$ , but chronic exposure to similar levels did have a number of effects on CO<sub>2</sub> exchange in hybrid poplar leaves. These included greater dark respiration rates in developing and young mature leaves and lower  $P_n$  in fully expanded leaves of all ages. The leaf maturation and aging processes themselves were well correlated with changes in CO<sub>2</sub> exchange. Age-related changes in  $P_n$ ,  $R_D$ , light-saturation, leaf Chl contents, and apparent quantum yield were relatively typical and followed the pattern of increasing activity during maturation and gradual decline in subsequent aging. However,  $P_n$  and  $R_D$  rates were extremely high for tree species, and similar to levels observed in many crops. Also, hybrid poplar leaves were capable of sustaining high rates of photosynthesis for extended periods of time; over 50 d at  $>20 \text{ mg dm}^{-2} \text{h}^{-1}$  and over 75 d at  $>10 \text{ mg dm}^{-2} \text{h}^{-1}$ .

Effects of exposure to low concentrations of O<sub>3</sub> were indicated by differences in age-related patterns of CO<sub>2</sub> exchange between control and ozone-treated plants. Until 7 d old, no differences in light-saturated  $P_n$  were observed between control and ozone-treated leaves, other than those due to differences in dark respiration. With increasing age, photosynthetic capacities and Chl contents of ozone-treated leaves declined more rapidly than in control leaves. Thus, although age-related patterns were similar for leaves in all treatments, they were accelerated in leaves exposed to O<sub>3</sub> and much of the decline in photosynthetic capacity of O<sub>3</sub>-treated leaves was associated with this accelerated leaf aging. Longevity is significantly shorter for ozone-exposed leaves of hybrid poplar 207 (14, 15), and O<sub>3</sub> is generally known to accelerate leaf senescence (9, 12). Although chronic exposure to O<sub>3</sub> did not affect light-saturated  $P_n$  until leaves were greater than 10 d old, effects at low light levels (on apparent quantum yield) or in the dark (on  $R_D$ ) were observed in leaves as young as 4 to

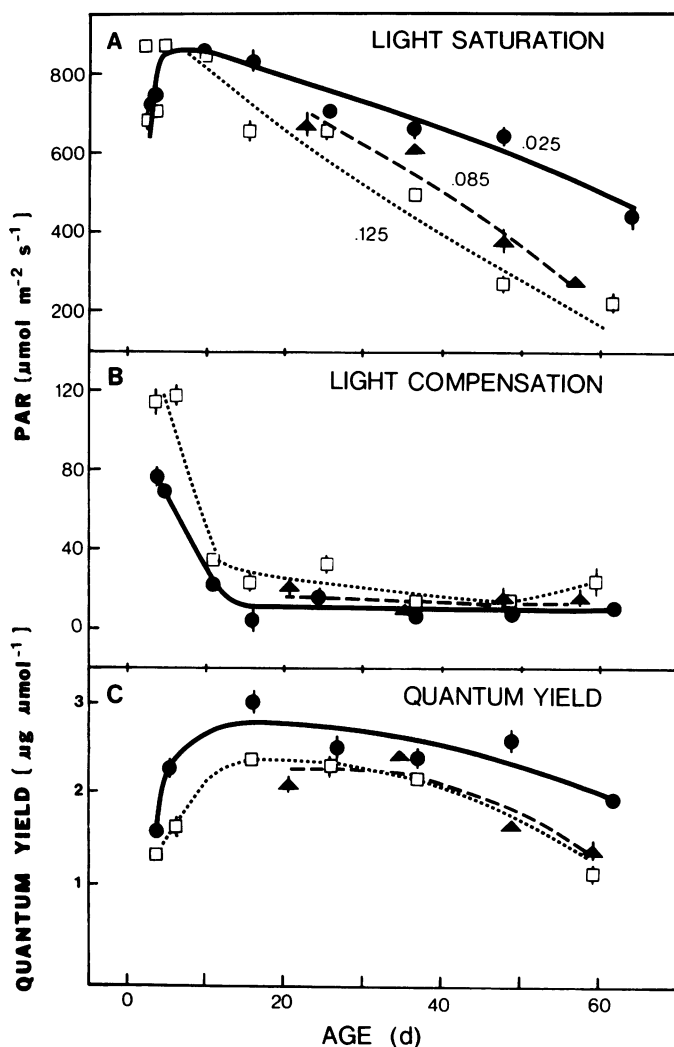


FIG. 2. Light saturation (A), light compensation (B), and apparent quantum yield (C) of variously aged hybrid poplar leaves chronically exposed to either 0.025 (●; control), 0.085 (▲), or 0.125 ( $\square$ )  $\mu\text{l l}^{-1}$  ozone;  $n = 2$  or 3.

6 d old. Effects of  $\text{O}_3$  exposure on  $R_p$  declined with increasing leaf age, and were minimal after leaves reached 35 d of age, while  $\text{O}_3$  effects on  $P_n$  were greatest in older leaves. Thus, effects of  $\text{O}_3$

on net photosynthesis and dark respiration were temporally unrelated, suggesting separate and distinct modes of action upon the mechanisms involved.

Other than accelerated leaf aging, what factors might have contributed to declining  $P_n$  in  $\text{O}_3$ -treated plants? Although light-saturated  $P_n$  was linearly correlated with Chl content, the data do not allow us to determine whether there was any causal relationship, and it is possible that both  $P_n$  and leaf Chl were independently, but similarly, affected by  $\text{O}_3$  treatments. What about potential indirect effects on  $P_n$  through stomatal perturbations? Extensive sampling of leaf diffusive conductance of leaves of all ages showed that the effects of  $\text{O}_3$  on  $P_n$  were not due to  $\text{O}_3$ -induced stomatal closure (14). For instance, depending on leaf age,  $\text{O}_3$ -treated leaves had lower  $P_n$  but either lower or higher diffusive conductance than control leaves. Also, although  $\text{O}_3$  exposure did not result in any discernible relationship between diffusive conductance and  $P_n$ , it did consistently reduce water use efficiency in leaves of all ages and at all light intensities (14). Thus,  $\text{O}_3$  probably affects the photosynthetic process itself, in some combination of the following ways.

Ozone-treated plants had lower apparent quantum yields than control plants, suggesting that  $\text{O}_3$  affects the photochemical reactions carried out by PSI and/or PSII. The effect was fairly constant over time, i.e. as leaves aged, the relative impact of  $\text{O}_3$  on apparent quantum yield did not increase. In contrast, as leaves aged, light-saturated  $P_n$  of  $\text{O}_3$ -treated leaves became increasingly reduced below  $P_n$  of control plants. Inasmuch as at light-saturating intensities neither photochemical reactions nor stomatal conductance (14) should be rate-limiting, chronic  $\text{O}_3$  exposure probably influences the actual reduction of  $\text{CO}_2$ , possibly by decreasing the content of RuBP-carboxylase, as has been found in alfalfa (13). In general,  $\text{O}_3$  effects on  $\text{CO}_2$  fixation could be either direct, or indirectly result from accelerated aging (due to  $\text{O}_3$  disturbing general metabolic processes), or both. More research is needed before this question can be answered.

Decreases in net photosynthesis following chronic exposure to low concentrations of  $\text{O}_3$  have been observed in a number of other species. Miller *et al.* (11) found significant declines in net photosynthesis of ponderosa pine seedlings treated for 60 d (9 h  $\text{d}^{-1}$ ) at 0.15  $\mu\text{l l}^{-1}$   $\text{O}_3$ . In our laboratory, recent studies with sugar maple, red oak, and soybean chronically exposed to either 0.09 or 0.13  $\mu\text{l l}^{-1}$   $\text{O}_3$  found significant decreases in net photosynthesis (Reich, Schoettle, and Amundson, unpublished data). In contrast to the results with poplar, very young  $\text{O}_3$ -treated soybean leaves showed immediate differences in  $P_n$  compared to controls, but never showed a difference in dark respiration. Perhaps increased dark respiration in poplar was due to increased maintenance

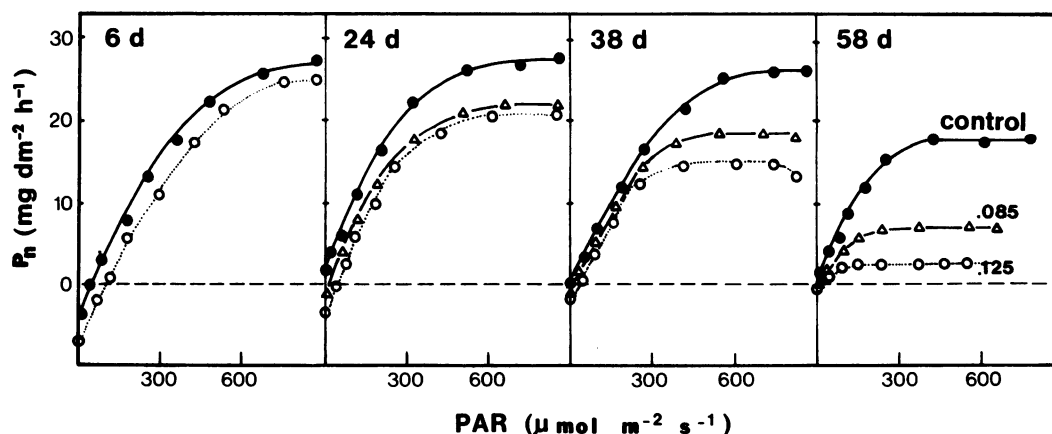


FIG. 3. Photosynthetic light response of variously aged hybrid poplar leaves chronically exposed to either 0.025 (●; control), 0.085 (△), or 0.125 (○)  $\mu\text{l l}^{-1}$  ozone. All curves represent mean values for two similarly aged leaves  $\pm 1$  SE.

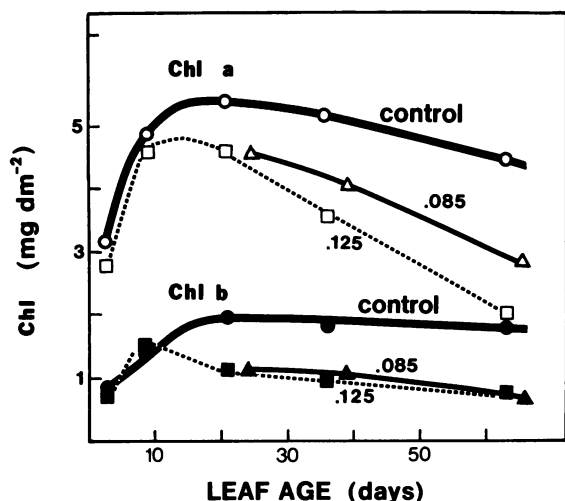


FIG. 4. Leaf Chl *a* (○, △, □) and *b* (●, ▲, ■) contents of variously aged hybrid poplar leaves chronically exposed to either 0.025 (○, ●; control), 0.085 (△, ▲), or 0.125 (□, ■)  $\mu\text{l l}^{-1}$  ozone. Values are means for six replicate samples in each age and treatment class. All SE bars fell within the symbols and thus are not shown.

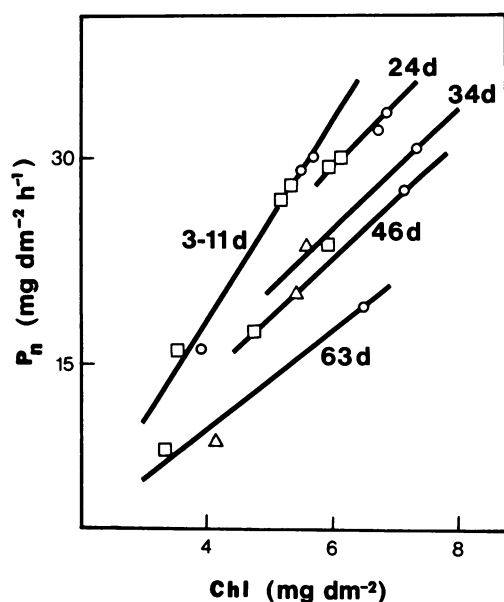


FIG. 5. Light-saturated net photosynthetic rates of leaves of various age classes in relation to leaf Chl *a* + *b* contents. Symbols are as in Figure 4.

which allowed ozone-treated leaves to maintain net photosynthesis rates similar to control leaves during much of the leaf maturation period.

Although no gas exchange work was done with poplar in the field, studies of growth and dry matter production using the same clone exposed to similar levels of ozone found similar, but greater, negative effects of O<sub>3</sub> exposure in the field than indoors (14). This was probably at least partially due to greater O<sub>3</sub> uptake rates by plants in the field, which had greater leaf diffusive conductances than plants grown indoors (14). Thus, results of this chamber study can probably be applied to plants grown in the field, especially since the greatest relative inhibition of  $P_n$  by O<sub>3</sub> was observed at the higher indoor light intensities, which were still only about one-third of full sunlight. Also, field studies in Europe and Japan (9, 12) with European hybrid poplar varieties support the results of this paper. Mooi (12) found accelerated

leaf aging in the Netherlands due to very low concentrations of O<sub>3</sub> (0.04  $\mu\text{l l}^{-1}$ ), and Kuno (9) observed significant reductions in net photosynthesis, leaf Chl contents, and growth due to ambient oxidant pollution near Tokyo, Japan. Finally, the results of this study underline the importance of examining the interacting effects of age and pollutant exposure upon whole leaf gas exchange. Without considering these interactions, any characterization of pollutant effects would be relatively incomplete.

## CONCLUSIONS

The results of these experiments are important in view of the significant concentrations of O<sub>3</sub> in ambient air in most areas of the developed world. Other studies carried out with chronic, low levels of O<sub>3</sub> have found similar negative effects and the role of O<sub>3</sub> as an agent of accelerated aging is well documented. From this study, it is apparent that chronic exposure to low levels of O<sub>3</sub> places a stress on the photosynthetic and respiratory systems of hybrid poplar leaves, resulting in increased dark respiration and decreased leaf Chl contents and net photosynthesis. Apparently, accelerated leaf aging due to O<sub>3</sub> exposure was at least partially responsible for declining net photosynthetic capacity in such leaves. Also, the differential timing of the greatest effects of O<sub>3</sub> exposure on net photosynthesis *versus* dark respiration implies the existence of separate modes of action. The results of this study suggest that more emphasis needs to be placed on the environmental problem of low level oxidant pollution, and that more attention needs to be paid to the physiological response of plants chronically exposed to low levels of O<sub>3</sub>.

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